

Abstract 108 – Table 1. Adjusted* Hazard Ratios for TGF- β 1 and OA Outcomes

Definition of OA Outcomes	n (knees with outcome)	Adjusted HR* (95%CI)	p value
Incident rOA (from baseline K-L <2 to K-L \geq 2)	103	1.10 (0.46-2.63)	0.83
Incident rOA (from baseline K-L <1 to K-L \geq 1)	94	1.04 (0.41-2.65)	0.93
Incident OST (from grade 0 to grade \geq 1)	94	1.41 (0.56-3.56)	0.47
Incident JSN (from grade 0 to grade \geq 1)	99	1.39 (0.50-3.88)	0.53
Progressive rOA (increasing \geq 1 grade from baseline K-L \geq 2)	86	1.36 (0.63-2.91)	0.44
Progressive rOA (increasing \geq 1 grade from baseline K-L \geq 1)	160	1.51 (0.82-2.79)	0.19
Progressive OST (increasing \geq 1 grade from baseline OST \geq 1)	76	0.55 (0.24-1.26)	0.16
Progressive JSN (increasing \geq 1 grade from baseline JSN \geq 1)	84	1.40 (0.64-3.03)	0.40

*Adjusted for age, race, gender, and body mass index.

6.1 \pm 1.3 years. There were no significant interactions by race or gender. The hazard ratios (Table) show no significant relationships between higher serum TGF- β 1 and incident rOA, OST, or JSN at the knee before or after adjustment. The odds of progressive rOA by either K-L definition were 40- 50% higher in association with higher serum TGF- β 1 levels, but this association was not statistically significant. There was an apparent reduction in OST progression with higher InTGF- β 1, also not statistically significant. **Conclusions:** Serum TGF- β 1 levels do not predict development of incident rOA, OST, or JSN in this longitudinal, population-based study including AA and Caucasian men and women. The finding of increased rOA K-L progression with higher serum TGF- β 1 levels did not reach statistical significance, even in this largest study to date of this biomarker, making it unlikely that serum TGF- β 1 will be a robust, stand-alone biomarker for future studies.

Reference

- [1] Nelson AE. Osteoarthritis Cartilage in press 2009; doi:10.1016/j.joca.2008.11.010.

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EX VIVO CHARACTERIZATION OF THREE NEW METALLOPROTEINASE-DERIVED NEOEPITOPIC BIOMARKERS OF TYPE II COLLAGEN

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Purpose: Type II collagen is the major collagen of the articular cartilage and it is gradually degraded during the pathogenesis of osteoarthritis (OA). Measurement of collagen type II degradation fragments may be associated with the progression of Osteoarthritis (OA). It is appreciated that aggrecan degradation is a combination of MMP and aggrecanase activity, resulting in a distinct and protease specific temporal release pattern, with aggrecanase activity at early time-points and MMP activity at later time-points. The release of collagen type II is less understood with respect to the temporal release pattern, and the different proteolytic proteases associated with that. We therefore identified three novel MMP-mediated collagen type II degradation fragments of collagen type II, and developed assays for that. We compared the release pattern to that of CTX-II.

Methods: Human type II collagen (purified) and human articular cartilage was digested with a mix of MMPs. The digested was analyzed on LC-MS/MS and peptide fragments were identified/mapped by Mascot database search. Three neoepitopic sequences were chosen, synthesized and used as immunogen. Fusions were made from the spleen of good-titer mice. After cloning and screening, the best clones were chosen and the monoclonal antibodies were purified and entered assay development. All assays were designed as follows: 1) biotin-peptid on streptavidin plates, 2) samples or standard, 3) Primary anti-

body, and 4) secondary HRP-antibody. Bovine explants cultures treated with proinflammatory cytokines were used for testing the biomarkers for native reactivity. Experiment including different proteases inhibitors were conducted to validated the origin of the new biomarkers. Established markers were measured in parallel for comparison. Furthermore; the antibodies were tested for localization by immunohistochemistry.

Results: Three assays were developed; CIIM538, CIIM1027 and CIIM1053. All three assays were optimized in respect to incubation buffer, time and temperature, as well as antibody concentrations. Following technical specification were established (on average): Range - 1-500 ng/ml, lower detection limit 0.2 ng/ml, dilution recovery 95-120%, intra- and inter-assay CVs < 6% and 11%, respectively. Measuring time-dependent release of the new biomarkers showed that the new type II collagen assays could profile the degradation of cartilage as a function of time. The releases of CIIM538 and CIIM1027 are significantly increased when treated with O+T for 13-15 days. These results show similar pattern to the release observed when we measure MMP-generated aggrecan fragments (342-G2). The third MMP-derived marker, CIIM1053, seems to displace the release to later time points, which resembles the release pattern of CTX-II. Furthermore the signal was inhibited when MMP inhibitor, but not other protease inhibitors, was added to the cultures.

Conclusions: We have developed three new biomarkers of type II collagen, specific for MMP neoepitopic sites in the helical region. These three assays display different release patterns; suggesting the use of these in profiling different stages or processes of cartilage degradation. The combination of several markers is needed for the understanding biological processes in preclinical settings, and these assay aid the understanding to distinct and relevant disease related processes. In a near future, we will introduce clinical sample to these assays in hope of developing important clinical tools.

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CIRCULATING LEVELS OF AGGREGANASE-DERIVED AGGREGAN FRAGMENTS ARE ELEVATED IN PATIENTS WITH RHEUMATOID ARTHRITIS, HOWEVER, CORRESPONDING MMP-DERIVED FRAGMENTS ARE NOT

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Purpose: Quantitative determination of cartilage degradation is important information for the assessment of patients with joint diseases, however, until now in vitro diagnostic products have failed to provide the necessary sensitivity and specificity. We therefore wanted to investigate the clinical utility of a series of new sandwich immunoassays quantitating different pools of aggrecan fragments carrying neo-epitopes generated by the proteolytic cleavage of aggrecanase and matrix metalloproteinases (MMPs).

Methods: Blood samples were collected from patients with rheumatoid arthritis (n=33) and healthy controls (n=33), serum was harvested and stored at -20°C until testing. Aggrecan fragments carrying the aggrecanase-derived neo-epitope NITEGE373 was determined using the G1-NITEGE373 ELISA, corresponding fragments carrying the 374ARGSV neo-epitope using the 374ARGSV-G2 ELISA, and finally total aggrecan was determined using the G1/G2 ELISA.

Results: We determined the concentration of G1-373NITEGE aggrecan fragments in human serum and found 989 ng/ml \pm 238 ng/ml (mean \pm SD) and 310 ng/ml \pm 42 ng/ml in patients with RA and controls, respectively. This corresponds to a 3-fold increase, which was highly significant ($p=0.004$). In parallel, we determined the concentration of the 374ARGSV-G2 fragments, which also is derived from aggrecanase-cleavage of aggrecan, however, no difference between RA and controls was detected. Finally, circulating levels of total aggrecan, as determined by the G1/G2 ELISA, was suppressed in RA patients vs controls ($p=0.0003$), whereas MMP-derived fragments determined in the 342FFGVG-G2 ELISA was similar in the two groups.

Conclusions: We conclude, that whereas the total circulating levels of aggrecan fragments carrying the G1 and/or G2 domains is suppressed in RA patients, then the serum concentration of the aggrecanase-derived aggrecan neo-epitopes, but not the MMP-mediated, are elevated in patients with RA. Circulating aggrecanase-mediated aggrecan fragments may be more sensitive than MMP-derived fragments to detect cartilage damage in RA.

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SERUM cartilage oligomeric matrix protein CONCENTRATION CAN PREDICT SUBSEQUENT MRI CHANGES OF EARLY KNEE OA

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Purpose: Serum measures of cartilage oligomeric matrix protein (COMP), a marker of cartilage degradation, may be a promising tool for evaluating OA severity or perhaps predicting disease progression. Here we use COMP to predict the changes of early knee OA detected by peripheral MRI during a two year follow up study.

Methods: 33 patients (8 men and 25 women) aged 45-65 years with chronic (>2 month duration) knee pain and diagnosed with early knee osteoarthritis were chosen and followed for 24 months. A blood sample was obtained at baseline and the serum concentration of COMP was determined by immunoassay. MRI scans (3D

Gradient Echo for cartilage evaluation) for the symptomatic knee were performed at baseline, 6 months, 12 months and 24 months later. A trained radiologist semiquantitatively graded cartilage on a scale of 0-4 in each of the medial tibia, lateral tibia, medial femur and lateral femur. The maximum score that can be obtained is 24. The baseline MRI cartilage score of all patients is ranged from 7 to 14.

Results: A significant, positive regression was found between baseline serum COMP concentration and the changes of MRI cartilage scores over 24 months ($R^2=44.5\%$, $P<0.001$).

Conclusions: These results suggest that higher COMP at baseline in patients with early OA could reflect increased cartilage degeneration over time relative to those with lower COMP levels. These findings support the hypothesis that a marker of cartilage degradation may predict future cartilage degeneration.

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ANALYSIS OF A BROAD SPECTRUM OF URINARY AND SERUM BIOMARKERS IN A LARGE COHORT OF PATIENTS WITH EARLY OA OF HIP AND/OR KNEE (CHECK): THE FIRST RESULTS

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Purpose: Biomarkers to diagnose OA in an early stage and/or to predict its course would be of great value. Therefore, we selected a broad spectrum of commercially available serum and urinary biomarkers representative of cartilage, bone and synovium metabolism for evaluation in the Cohort Hip & Cohort Knee (CHECK). This cohort consists of 1002 participants with pain and/or stiffness of knee and/or hip, aged 45-65 yrs, and who had never or no longer than 6 months before visited their physician for these symptoms for the first time. Hip and knee radiographs as well as clinical parameters and multiple questionnaires are evaluated at regular intervals. Furthermore, blood (plasma and serum; s) and urine (u) samples (multiple aliquots of all three) are collected at 0, 2, 5, 8, and 10 yrs. Samples are obtained in 10 medical centers and processed in the same central laboratory, all according to strict protocols. Biomarker measurement in these samples provides a unique opportunity to study the diagnostic and prognostic properties of biomarkers in early-stage OA. Baseline biomarker measurement has recently been completed. The data set is huge, specifically in combination with all patient characteristics and longitudinal radiographic and clinical data. Before relations are calculated we have to be sure about the quality of the biomarker data set. This abstract covers this issue.

Methods: Baseline samples were thawed for the first time. Commercially available ELISA assays were performed to measure uCTX-II, uCTX-I (ImmunoDiagnostic Systems Ltd.), uNTX-I (Wampole Laboratories), sCOMP (Anamar Med AB), sOC (IDS), sHA (Corgenix Inc.), sPILINP (Millipore Corp.), sCS846, and sC1,2C (IBEX) and RIA assays for sPINP and sPILINP (Orion Diagnostica). All kits were purchased without commercial involvement and for each biomarker kits were from the same badge.

A serum and urine pool were created from several OA patients and aliquoted. At each of the assay days an aliquot was thawed and included at multiple places in the assay plates. Each biomarker was measured in all samples by the same technician in 8 days in a period of 6 wks using 14 kits.

Results: Quality control as was performed by using a randomly distributed pool sample, controls as supplied by manufacturers, and blank samples in-between patient samples was unremarkable, except for the COMP assay. The COMP assay's pool sample showed a remarkably variable biomarker concentration within and between assay plates.

